PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Sinan Tas
Application No. : 10/682662
Filed : October 9, 2003
For : USE OF CYCLOPAMINE IN THE TREATMENT OF PSORIASIS
Examiner : Frederick Krass
Group Art Unit : 1614

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Docket No. : M67.2H-11334-US01

DECLARATION OF DR. SINAN TAS

SINAN TAS states:

I. Background and Expertise

1. I am one of the inventors of the above titled application. I am a Medical Doctor (M.D., Hacettepe University, Ankara, Turkey, 1976). I have a Ph.D. in Experimental Pathology (University of California at Los Angeles, 1980). I have also received additional educational certificates and diplomas detailed in my Curriculum Vitae, attached as **Exhibit A.**

2. My professional experience includes numerous clinical and teaching positions at academic institutions in the United States, Turkey and Kuwait working in the fields of pathology, biochemistry, clinical biochemistry and molecular genetics. These positions are also listed in **Exhibit A.**

3. In the course of my work I have assisted in developing a first year medical curriculum at Inönü University, Malatya, Turkey and Ph.D. programs in oncology and biochemistry at Dokuz Eylül University, Izmir, Turkey. I have also done extensive research in the fields of molecular and cellular mechanisms of aging, the structure and regulation of the eukaryotic genetic material and in tumor biology. I have numerous publications in those fields which are also listed in **Exhibit A.**

4. Based on my training and experience, I am qualified to evaluate state of the art in the area discussed below.

II. Level of Skill in the Art - Inhibition of Hedgehog/Smoothened Signaling By Cyclopamine and By Non-Cyclopamine Molecules

5. The gene for Hedgehog (Hh) was identified first in Drosophila through its effects on larval patterning. Homologues of Drosophila Hh were later found in higher organisms. Similar to the situation in Drosophila, the homologues in higher organisms were found to be secreted proteins that affect embryonal patterning and induce differentiation of various cell types during development. How Hh signaling occurs was determined originally mostly in Drosophila. A transmembrane protein, Patched, was found to act as a receptor for Hh. When not liganded by Hh, Patched was shown to act to inhibit the intracellular signal transduction by another transmembrane protein, Smoothened (Smo). Binding of Hh to Patched was determined to relieve the inhibitory effect of Patched on Smo. The relieved Smo was found to initiate a series of events in the cell, resulting in the accumulation of full length Cubitus interruptus (Ci) protein which appeared to directly mediate transcriptional activation of several Hh target genes. It was also described that a proteolytically processed (lower molecular mass) Ci can act as a repressor of transcription of a number of genes. As mentioned on page 2, lines 2-21 of the international application incorporated to the present application (PCT/TR01/00027, published by WIPO as WO 02/078703), general features of Hh/Smo signaling described in Drosophila were found later to be similar in the more advanced organisms. However, a larger number of gene products were identified in the more advanced organisms to take part in Hh/Smo signaling. For example, human and mouse were described to have three homologues of the single Hh of Drosophila (Sonic hedgehog, Indian hedgehog, Desert hedgehog) and three homologues of the single Ci of Drosophila (the Gli family proteins Gli1, Gli2, Gli3).

6. Cyclopamine (11-deoxojervine) and related teratogenic *Veratrum* alkaloids (e.g. jervine) had long been known in prior art to produce cyclopia and other developmental abnormalities similar to the human holoprosencephaly in the offspring of pregnant animals exposed to said compounds [see e.g. *Keeler RF, Proceedings of The Society for Experimental Biology and Medicine* 1975;149:302-306 (**Exhibit B**)]. Cyclopia was found to be a feature also in the Zebrafish embryos lacking expression of the Zebrafish homologue of Hh at the neural plate midline of developing brain [*Krauss S et al, Cell* 1993;75:1431-1444 (**Exhibit C**)]. Similarly, the loss-of-function mutations of the gene for Sonic hedgehog (Shh) were described to cause cyclopia and holoprosencephaly in human [*Belloni E et al, Nature Genetics* 1996;14:353-356 (**Exhibit D**); *Roessler E et al, Nature Genetics* 1996;14:357-360 (**Exhibit E**)] and mouse [*Chiang C et al, Nature* 1996;383:407-413 (**Exhibit F**)]. In accord with these earlier findings, the scientific publications cited on page 3, lines 19-22 of the specification of present application described in 1998 that cyclopamine as well as jervine can be used to specifically inhibit Hh/Smo signaling [*Cooper MK et al, Science* 1998;280:1603-1607 (**Exhibit G**), see abstract "*... these compounds specifically block the Sonic hedgehog(Shh) signaling pathway*"; *Incardona JP et al, Development* 1998;125:3553-3562 (**Exhibit H**), see Abstract "*...cyclopamine-induced teratogenesis is due to a ... direct antagonism of Shh signal transduction*"]. Specificity of the inhibition of Hh/Smo signaling by these teratogenic *Veratrum* alkaloids was deduced from the fact that they selectively inhibited the differentiation events induced by Shh (*Cooper MK et al,*

ibid; *Incardona JP et al, ibid*). Indeed, the same Shh-induced differentiation events inhibited by cyclopamine and jervine were shown earlier to be selectively inhibited by affinity purified antibodies binding and blocking Shh [*Ericson J et al, Cell* 1996;87:661-673 (**Exhibit I**)]. Thus, cyclopamine's inhibition of Hh/Smo signaling specifically was a well known biological effect and it was described in prior art that non-cyclopamine compounds can also be used for that purpose.

7. Further examples of non-cyclopamine compounds were also described to be suitable to inhibit Hh/Smo signaling specifically. Signaling by Shh and by other Hh family proteins was shown to be specifically inhibited by monoclonal antibodies as well as by other molecules recognizing Hh proteins [see *Ericson J et al, Cell* 1996;87:661-673 (**Exhibit I**); *Goodrich LV et al, Neuron* 1998;21:1243-1257(**Exhibit J**); *Chuang PT et al, Nature* 1999;397:617-621 (**Exhibit K**); *Wang LC et al, Journal of Investigative Dermatology* 2000;114:901-908 (**Exhibit L**); *Treier M et al, Development* 2001;128:377-386 (**Exhibit M**)]. For example, the scientific publication cited on page 3, lines 22-25 of the specification in present application refers to the inhibition of Hh/Smo signaling by cyclopamine and describes that non-cyclopamine molecules can also be used to inhibit Hh/Smo signaling similarly [see *Goodrich LV et al, Neuron*, 1998;21:1243-1257 (**Exhibit J**)]. Still other categories of non-cyclopamine molecules had also been described in prior art to specifically inhibit Hh/Smo signaling as demonstrated e.g., by assaying for the effects on Gli family proteins (intranuclear executors of Hh/Smo signaling) [see *Williams KP et al, Journal of Cell Science* 1999;112:4405-4414 (**Exhibit N**)]. In summary, inhibition of Hh/Smo signaling specifically by cyclopamine and by various non-cyclopamine compounds was well known in prior art and the specification of application mentions this fact.

8. Methods of assaying Hh/Smo signaling and methods of synthesis of libraries of related molecules (e.g. combinatorial chemistry) were common knowledge in prior art. After reading the present application, a person of ordinary skill in art could readily recognize identity of a non-cyclopamine molecule suitable for use in the described invention and he/she could obtain further such molecules by methods routinely used in prior art. For example, total chemical synthesis of teratogenic *Veratrum* alkaloids and derivation of these molecules from one another had been described in prior art [see e.g. *Kutney JP et al, Canadian Journal of Chemistry* 1975;53:1796-1817 (**Exhibit O**)] and a molecule derived from cyclopamine had been used to inhibit Hh/Smo signaling and to obtain the previously known technical effect of the inhibition of cell proliferation [*Taipale J et al, Nature* 2000;406:1005-1009 (**Exhibit P**)].

9. Numerous naturally occurring and synthetic non-cyclopamine molecules with and without a cyclopamine core were well known in prior art to be suitable for specifically inhibiting Hh/Smo signaling. Specification of application cites several examples of prior art publications describing such uses of non-cyclopamine molecules. The specification also indicates that molecules can be synthesized so as to possess the disclosed biological effects of cyclopamine (see e.g. page 17, lines 22-25). Thus, it is clear to a person skilled in the art that a pharmaceutically acceptable compound that specifically inhibits Hh/Smo signaling is suitable for use in the described method of treatment. A molecule defined functionally as a molecule that possesses the biological effects of cyclopamine and inhibits Hh/Smo signaling

specifically is a clearly defined molecule. The skilled person would have no uncertainty whether a particular non-cyclopamine molecule is within or out of the defined category of molecules. The specification teaches directly that a molecule other than cyclopamine exerting the biological effects of cyclopamine is suitable for use in the described method of treatment.

10. Finally, as a more general observation, I have found that defining a category of compounds in terms of the particular function that they carry out can be more effective than structural definitions in enabling identification of useful compounds for specific medicinal use, provided that the defined function is clear and effective compounds are available for comparison. Consider, for example, the investigations starting in 1960s and leading to the description in 1998 of the teratogenic *Veratrum* alkaloids as specific inhibitors of Hh/Smo signaling. As reviewed by *R.F. Keeler* in *Teratology* 1970;3:169-174 **(Exhibit Q)**, detailed physical and chemical features and molecular structure of numerous *Veratrum* alkaloids were determined by 1970s. Correlation between structure and teratogenicity was also found. Rigid positioning of the piperidine ring with respect to the steroid system in cyclopamine and in other teratogenic *Veratrum* alkaloids was identified as a distinguishing feature of them from non-teratogenic alkaloids. However, in the absence of an understanding of basically what these compounds do to produce the teratogenic effects, the detailed physical-chemical descriptions that had become available were of little use to identify further such compounds even in *Veratrum* and the contemplations were in the wrong track (see e.g. page 171 in *Keeler RF, ibid*). On the other hand, the realization that the teratogenic *Veratrum* compounds and various other compounds that produce the observed specific teratogenic effects have all in common the specific inhibition of Hh/Smo signaling opened up the way to the identification of numerous new compounds suitable to produce a previously known technical effect. Thus, it simply is untrue that a clear functional definition would be insufficient and would not enable a skilled person to practice the invention or to understand its metes and bounds.

III. Certification - 37 CFR §1.68

11. The undersigned having been warned that wilful false statements and the like are punishable by fine or imprisonment, or both under the provisions of 18 U.S.C. §1001 and may jeopardize the validity of the application or any patent issuing thereon, hereby declares that all statements made of his own knowledge are true and that all statements made on information and belief are believed to be true.

Date: December 13, 2007 By: SinanTas
Sinan Tas

LIST OF THE EXHIBITS

Exhibit A: Curriculum Vitae, Sinan Tas

Exhibit B: Keeler RF, *Proceedings of the Society for Experimental Biology and Medicine* 1975;149:302-306.

Exhibit C: Krauss S et al, *Cell* 1993;75:1431-1444.

Exhibit D: Belloni E et al, *Nature Genetics* 1996;14:353-356.

Exhibit E: Roessler E et al, *Nature Genetics* 1996;14:357-360.

Exhibit F: Chiang C et al, *Nature* 1996;383:407-413.

Exhibit G: Cooper MK et al, *Science* 1998;280:1603-1607.

Exhibit H: Incardona JP et al, *Development* 1998:125:3553-3562.

Exhibit I: Ericson J et al, *Cell* 1996;87:661-673.

Exhibit J: Goodrich LV et al, *Neuron* 1998;21:1243-1257.

Exhibit K: Chuang PT et al, *Nature* 1999;397:617-621.

Exhibit L: Wang LC et al, *Journal of Investigative Dermatology* 2000;114:901-908.

Exhibit M: Treier M et al, *Development* 2001;128:377-386.

Exhibit N: Williams KP et al, *Journal of Cell Science* 1999;112:4405-4414.

Exhibit O: Kutney JP et al, *Canadian Journal of Chemistry* 1975;53:1796-1817.

Exhibit P: Taipale J et al, *Nature* 2000;406:1005-1009.

Exhibit Q: Keeler RF, *Teratology* 1970;3:169-174.